

FIG. 1. Sensitivity of the PCR assay. Shown are the results of PCR amplification of the serially diluted *Z. mangeloni* (DD8) DNA analyzed on agarose gels. DNA was extracted from parasite cultures and amplified as described in Materials and Methods. Lane M, 1 kb Ladder (Gibco BRL); Lane 1, 10 pg of DNA; Lane 2, 1 ng of DNA; Lane 3, 10 pg of DNA; Lane 4, 1 pg of DNA; Lane 5, 10 fg of DNA; Lane 6, 1 fg of DNA.



Probe: Ld Ind kDNA

Human DNA: $\xrightarrow{100 \text{ ng}}$

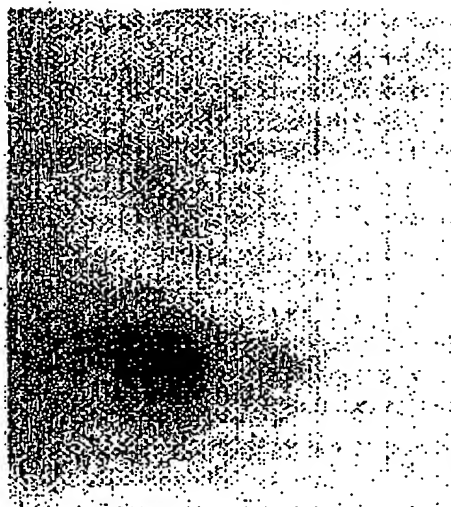
Primer Set: Ldl1 & 2

Amt. Ld Ind DNA: $\begin{matrix} 0.01 \text{ ng} \\ 0.1 \text{ ng} \\ 1 \text{ ng} \\ 10 \text{ ng} \end{matrix}$

(Kb)

0.87 —

0.6 —

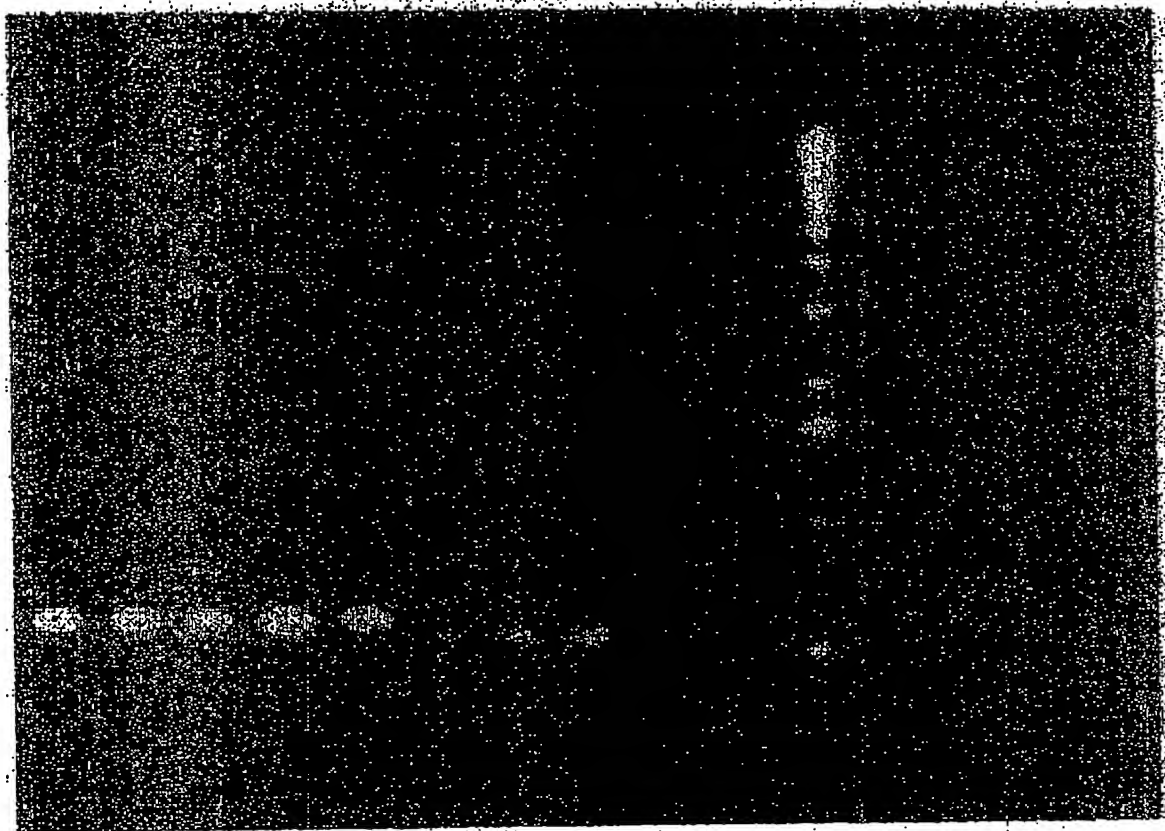


1 2 3 4

FIG. 2. Sensitivity of PCR amplification of *Leishmania* kDNA followed by Southern blot analysis. The PCR contained 100 ng of human genomic DNA and the indicated amount of total DNA from *L. donovani* DD8. The PCR product was probed with parasite kDNA and exposed for about 1 h. Lane 4 represents a PCR containing only human DNA as a control.



1 2 3 4 5 6 7 8 9 10 M 11 12 13



00 bp —

FIG. 3. Amplification of parasite DNA from various strains and isolates of *Leishmania*. DNA (1 ng) isolated from parasite cultures was subjected to PCR and analyzed. Lane 1, *L. donovani* AG83; lane 2, *L. donovani* DD8; lane 3, *L. donovani* HCB8; lane 4, *L. donovani* CB6; lane 5, *L. donovani* HCB 7 (PKDL origin); lane 6, *L. donovani* WR683; lane 7, *L. donovani* WR684; lane 8, *L. donovani* infantum; lane 9, *L. tropica* WR683; lane 10, *L. major* I.V. 39; lane M, 1-kb ladder; lane 11, *Plasmodium*; lane 12, *M. leprae*; lane 13, *M. tuberculosis*.



M 1 2 3 4 5 6 7 8 9 10 11



— 600 bp

FIG. 4. DNA amplification from recent field isolates of KA and KDL. DNA (1 ng) extracted from cultures of parasite isolates was used for PCR amplification. Lanes: M, 1-kb ladder; 1, KA-1; 2, KA-2; 3, KA-3; 4, KA-4; 5, KA-5; 6, PK-1; 7, PK-2; 8, PK-3; 9, PK-4; 10, PK-5; 11, isolate from a patient with cutaneous leishmaniasis.

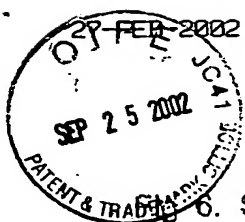


M 1 2 3 4 5 6 7



600 bp

FIG. 5. PCR assay with clinical samples of KA and PKDL. DNA (100 ng) isolated from clinical samples was used for PCR amplification. Lane M, 1-kb ladder; lane 1, KA (bone marrow); lane 2, KA (blood); lane 3, malaria (blood); lane 4, tuberculosis (blood); lane 5, control from the area of endemicity (blood); lane 6, PKDL (skin lesion); lane 7, leprosy lesion).



6. Sequence of PCR products with DNA isolated from *L. donovani* DD8 strain, isolates and clinical samples of KA and PKDL.

1 gaattcgccg aaaaatgacc gaaaatgggc aaaaaacca aacttttctg gtccctcggg
61 tagggcggtt ctgcgaaaac cgaaaaatgg gtgcagaaat cccgttcaaa aaatagccca
121 aaatgccaaa aatcggtccc gaggggggaa actggggggt ggtgtaaaat agggctcggg
181 ggaggggaaa ctgcggggtc ggacgtgtgt ggatatggcc tgggtgggga ctttggagt
241 ggttgtactt gtatgggggt tggacctg cttgggggtt ggggggtggg gtgggaaagg
301 ggtcggcgct atttggagt acgttggctc ttttgataat tgatatattg tctaaactgg
361 attgggttcg ctggatatac gttgggttgg ttggatttgg attggatttg gatrtgtac
421 ggggttggag gcttgarttg ggggtgagga gtttgtgggg atagttttgg atgttagtat
481 ggastgtagc ttcctttaat ataaatatta gttggggcrg ttgcattagt ttgttcacg
541 ggagtagcct caggacttta ggcgggagat actatattat cggtagtata atactataag
601 tatacggtat agatataagt taattgtagt atattgtaga tctatgttac agtgtatagt
661 ctatgaactt actagatata atttgtattt gatgctatag tgctactgat agagtgtacc
721 tatcactagt atagacgtag ctgaagctcc ctaaatgggt gggaaatgggt gtgaggggtg
781 gaagagacac cg